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INTRODUCTION

Colorectal cancer (CRC) is the 4th most common cancer in the United States and worldwide and ranks 2nd in cancer-related deaths (1). Although there is an overall improvement in

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Abstract

Aim: Colorectal cancer (CRC) is the 4th most common cancer, and tumor tissue has a higher amount of myeloperoxidase (MPO)-positive cells than normal tissue. We aimed to compare the parameters of myeloid and lymphoid cells and MPO levels in the venous blood of patients diagnosed with CRC and healthy volunteers. **Materials and methods:** The patient group in the study consisted of people who were diagnosed with CRC and had not yet received local or systemic treatment. The control group was selected from non-cancer patients. The enzyme-linked immunosorbent assay was used for the quantitative determination of MPO in serum.

Results: A total of 56 patients were included in this study. The mean MPO level of the patient group was significantly lower than that of the control group (3.59, 5.2 pg/mL, $p=0.007$). As a result of the receiver operating characteristics curve analysis, the cut-off point obtained for MPO was determined to be 3.66. There were significantly more CRC patients in the group with an MPO value of 3.66 and below ($p=0.001$; $p<0.01$). An MPO value of 3.66 or below increased the risk of CRC by 8.13 times.

Conclusions: In our study, the serum MPO level was significantly lower in the CRC patients, suggesting that immune system cells in CRC tend to behave differently in cancerous tissue and the general circulatory system. In the light of future studies on this subject, simple and easily reproducible determination of plasma MPO level may help to predict the prognosis, treatment planning, response to treatment, and efficacy of immunotherapy in CRC.

Keywords: Colorectal cancer, myeloperoxidase, predictor

the incidence of CRC and mortality rates associated with this disease, research has shown that the incidence of CRC is actually increasing in the population under 50 years of age (2). According to the researchers' estimates, by 2030, colon and rectal cancer rates under the age of 35 will increase by 90% and 124.2%,

respectively (3). The currently accepted approach is that the TNM stage and tumor status at the time of initial diagnosis are suggestive and guiding for prognosis in CRC patients (4). Despite all this, none of these have independent prognostic value for this patient group (5,6). Therefore, new parameters are needed to predict disease progression and efficacy of treatment in CRC. The need for these new parameters is gaining importance, especially to predict the effectiveness of new treatments such as immunotherapy.

Myeloperoxidase (MPO), a lysosomal enzyme, is intensely produced in the early maturation phase of neutrophilic granulocytes (NGs) (7). MPO is involved in the production of hypochlorous acid and does this by catalyzing hydrogen peroxide and chloride. It also forms tyrosyl radicals by oxidizing tyrosine. These two compounds (hypochlorous acid and tyrosyl radicals), which are formed by the effect of MPO, are cytotoxic to many microorganisms. Also, after MPO plays a role in granulocyte activation, it induces granulocyte apoptosis (8,9). Studies have shown that colorectal tumor tissue has a higher amount of MPO-positive cells than normal tumor-free tissue (10,11).

In this study, our aim is to compare the parameters and MPO levels of myeloid and lymphoid cells in the sera of patients diagnosed with CRC and healthy volunteers.

MATERIAL AND METHODS

This prospective study was approved by the Ethics Committees of our hospital (approval number: 2018/351) and was conducted in accordance with the ethical standards of the Declaration of Helsinki. Written consent was obtained from all participants in the study. Patients who underwent colonoscopy between March 2018 and March 2019 and were diagnosed with colorectal carcinoma based on the biopsy results but had not yet undergone any local or systemic therapy were included in the study as the CRC group. The control group was selected from patients without cancer of similar age and sex to the patient group. All the patients' height and weight were recorded, and additional diseases such as diabetes and hypertension were questioned. The body mass index (BMI) is calculated as weight in kilograms divided by the square of height in meters. Patients under 18 years of age, those with active infections or chronic inflammatory diseases including diabetes mellitus, smokers, obese patients, pregnant women, and patients who were unable or unwilling to provide consent were not included in the study.

Before surgery and anti-cancer treatment, 2 ml of venous blood was taken from the patients to measure the complete blood count and measure the plasma MPO level. Quality assurance has been applied to demonstrate the accuracy and precision of the tests. The complete blood count analysis was undertaken using the ADVIA 2120i hematology autoanalyzer

(Siemens Healthcare Diagnostics, Erlangen, Germany). The enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of MPO in serum (ELISA Kit Booklet, KTE61560, Abbkine).

Statistical analysis

For the statistical analysis, we used the Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) software. To compare the two groups for variables showing normal distribution, we used Student's t-test, and for those who did not show a normal distribution, we used the Mann-Whitney U test. One-way variance analysis was applied to compare more than two groups with normal distribution; the Bonferroni test was used to determine which group caused a significant difference. Pearson chi-square test was used to compare qualitative data, and Pearson and Spearman's correlation analysis was used for quantitative variables. Diagnostic screening tests such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and receiver operating characteristic (ROC) curve analysis were used to determine the cut-off values of the investigated parameters. $p < 0.05$ was considered significant.

RESULTS

This study included 56 patients with CRC, of whom 43 were male (76.8%) and 13 were female (23.2%) with a median age of 64 years. Twenty age- and sex-matched individuals without cancer were included in the control group. The median BMI of the patient group was significantly higher than that of the control group (26.07 and 23.27, respectively; $p = 0.001$).

The mean MPO level of the patient group was significantly lower than that of the control group (3.59 ± 2.26 and 5.2 ± 2.1 pg/mL, respectively; $p = 0.007$). In addition, in the CRC group, the white blood cell count, neutrophil, and neutrophil-lymphocyte ratio (NLR) values were higher, while the lymphocyte, hemoglobin, and hematocrit values were lower compared to the controls (Table 1).

As a result of the ROC curve analysis, the cut-off point of MPO was determined to be 3.66, at which it had 58.93% sensitivity, 85% specificity, 91.7% PPV, and 42.5% NPV. The area under the obtained ROC curve was determined as 71.4%, with a standard error of 6.2%. When grouped according to the cut-off value, there were significantly more patients with CRC in the group with an MPO value of 3.66 and below ($p = 0.001$; $p < 0.01$). Having an MPO value of 3.66 or below increased the risk of CRC by 8.13 (CI 95%: 2.133-30.984) times (Figures 1,2).

In the CRC group, there was no significant difference in the MPO levels according to cancer stage and tumor size ($p = 0.637$ and $p = 0.769$, respectively) (Table 2).

Table 1. Distribution of MPO and Blood Parameters According to Groups

	Group		P
	Control (n = 20) Range (Median) Mean $\pm$ SD	CRC (n = 56) Range (Median) Mean $\pm$ SD	
MPO pg/mL	1.78-8.94 (4.55) 5.2 $\pm$ 2.1	0.35-10.46 (3.21) 3.59 $\pm$ 2.26	^a 0.007**
WBC x 10 ⁹ /L	4850-19150 (6840) 8212.5 $\pm$ 3310.3	4220-19920 (9780) 9933.21 $\pm$ 3214.23	^a 0.045*
Neutrophil x10 ⁹ /L	2370-16810 (4045) 5021 $\pm$ 3342.36	2860-15980 (7190) 7450.54 $\pm$ 3113.83	^b 0.001**
Neutrophil (%)	45.6-90.2 (54.85) 58.27 $\pm$ 12.56	41.7-93.8 (77) 73.7 $\pm$ 13.69	^b 0.001**
Lymphocyte x10 ⁹ /L	660-4590 (2170) 2321 $\pm$ 968.92	210-4400 (1470) 1566.96 $\pm$ 1042.57	^a 0.006**
NLR	1.03-16.35 (1.68) 2.99 $\pm$ 3.84	0.94-41.1 (5.08) 9.03 $\pm$ 9.18	^b 0.001**
Lymphocyte (%)	1.7-44.3 (22.6) 21.83 $\pm$ 14.22	2.6-44.4 (15.65) 17.47 $\pm$ 11.05	^a 0.166
Hemoglobin (g/dl)	10.9-14.8 (12.9) 12.99 $\pm$ 1.11	7.3-17.2 (11.4) 11.28 $\pm$ 2.08	^a 0.001**
Hematocrit (%)	34.4-44.3 (39) 39.42 $\pm$ 3.11	23.9-51.6 (35) 34.88 $\pm$ 5.52	^a 0.001**

^aStudent's t-test^bMann-Whitney U test

*p < 0.05

**p < 0.01

SD = standard deviation, MPO = myeloperoxidase, WBC = white blood cells, NLR = neutrophil-lymphocyte ratio

Table 2. Relationship of MPO with Disease Stage and Tumor size

		MPO				P
		Mean	SD	Median	Min- Max	
Cancer stage	4	3.59	1.99	3.544	0.40-7.16	^a 0.637
	<4	3.52	2.37	2.944	0.34-10.46	
Tumor	1-2	3.94	2.54	3.474	0.38-10.46	^b 0.769
Size	3	3.49	1.79	3.184	0.34-5.83	
	4	3.40	2.324	3.024	0.40-8.92	

GFR: Glomerular filtration rate, CRP: C-reactive protein, MNA: Mini nutritional assesment

Parameters were expressed median (Q1-Q3)

*p<0.05 was considered significant for statistical analyses

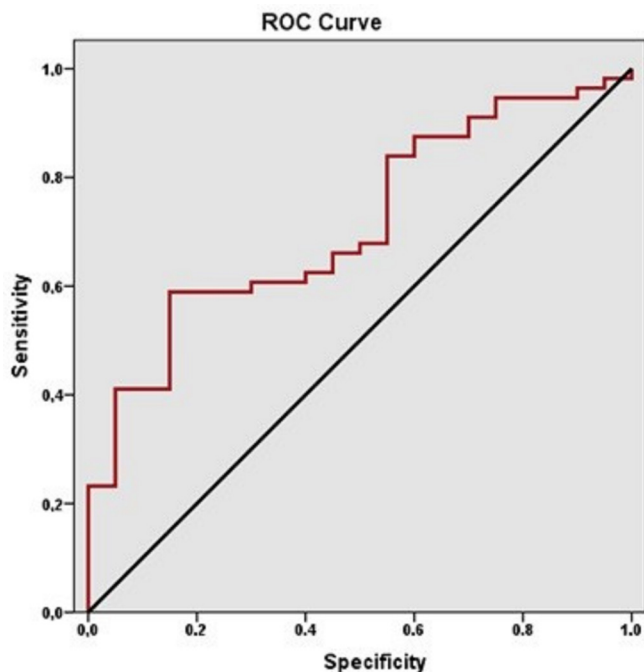


Figure 1. ROC curve plot for MPO by Groups

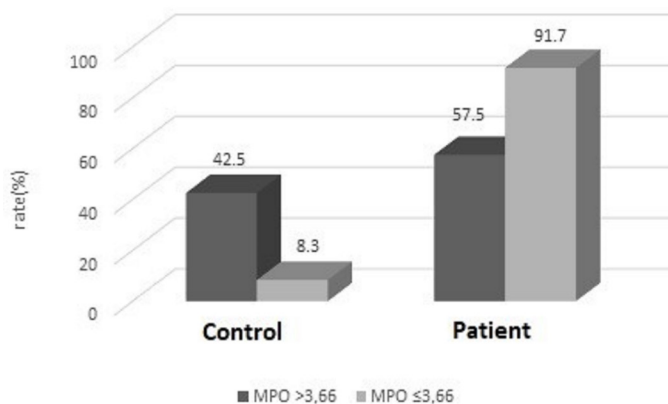


Figure 2. Distribution of MPO Levels in Groups

DISCUSSION

NGs are the most abundant white cells in the circulation and are the most important component of the primary defense mechanism against infection. Studies have shown that the properties of immune cells that infiltrate the tumor are associated with the development and progression of cancer (12-14). In particular, it is considered that an excess of intratumoral myeloid cells promotes tumor progression and is therefore associated with poor disease outcomes (15).

The mechanism underlying the infiltration of tumor tissue by MPO + cells in CRC, the molecular background associated with it, and their relationship to disease prognosis is not yet clear. Ongoing antitumoral adaptive responses due to chemokines

produced by activated T cells may be related to the course of the tumor. Alternatively, this situation can be explained by chemokines released by tumor cells triggering granulocyte production (16). In CRC, NGs that reach the tumor tissue and infiltrate it can directly show antitumor activity by the effect of cytokines, chlorinated oxidants, and enzymes, especially MPO (17). In a study by Droese et al. (18), a high amount of MPO + cells in tumor tissue in CRC was shown to be independently associated with a favorable prognosis. In our study, we did not determine the MPO level in tumor tissue again since we know that its presence has already been proven in many studies. Rather, we focused on the MPO level in venous blood. The accumulation of granulocytes at the tumor site and their continuous activation there is beneficial both as a natural process and in the efficacy of immunotherapy in CRC patients, and there are studies supporting this (19).

MPO can be used as a convenient and valuable biomarker for the identification and quantification of active neutrophils. MPO is a marker of activated leukocytes and is associated with the generation of reactive oxygen species (ROS). Their activities in neoplastic tissue are increased compared to normal tissue, suggesting that activated leukocytes play a possible role in increased oxidative stress in cancerous tissue (20).

It has been proven that ROS originating from neutrophils can cause a host of cancer-causing damage to DNA. It contributes to cancer formation by many mechanisms such as point mutations in DNA, error formation in DNA during replication, and genetic instability (21,22). Moreover, studies have shown that CRC with microsatellite instability has a much higher MPO-positive cell infiltration than microsatellite-stable CRC. These studies show that genetic changes can affect neutrophil infiltration on their own. In other words, the role of inflammation and immunological response in tumors with microsatellite instability is more pronounced (10,23,24).

It has now been proven that increased NGs and MPO + cells are seen in cancerous tissue in CRC. Similarly, in our study, the neutrophil level and NLR were found to be significantly higher in CRC patients ($p = 0.001$ and $p = 0.001$, respectively). Our patients were newly diagnosed, had not yet undergone surgical resection, or received any cancer-directed treatment such as conventional chemotherapy, targeted therapy, and immunotherapy. However, the serum MPO level was found to be significantly lower in the CRC group. This indicated that immune system cells in CRC tend to behave differently in cancerous tissue and in the general circulatory system. Considering that MPO is effective in the induction of granulocyte apoptosis following granulocyte activation, the association between a low MPO level and elevated neutrophil and NLR in the circulatory system may be due to this mechanism. The types and density of cells that increase in CRC not only predict the survival of patients but also affect the response of the tumor to treatment. This provides hope that cells with increasing density in tumors can be used as clinical biomarkers (25-29).

The limitation of our study is that the MPO level was examined only once in the patients, and the relationship between the response to treatment and the MPO level was not examined. Focusing on this relationship in subsequent studies can guide treatment choices in CRC.

CONCLUSION

In conclusion, the prognosis of CRC depends on cancer cell-specific features such as existing mutations, microsatellite instability, epigenetic features, the interaction of malignant cells, and the tumor microenvironment (6). All these parameters not only allow for the predicting of the prognosis but also help predict treatment planning, response to treatment, and efficacy of immunotherapy. However, it is not always easy and accessible to analyze tumor tissue. Tumor tissue analyzed at the time of diagnosis may not be useful for the clinician in the later stages of the disease due to changes in the characteristics of the tumor over time and the development of different resistance mutations in tumor cells with the effect of treatments received. However, analyses performed on venous blood samples, which can be easily accessed, can better reflect the current situation. It is also an advantage that the process is easy to apply and easy to repeat. Thus, it can help us obtain up-to-date data on the immune response status of the tumor and the patient during treatment. In the future, investigating the relationship between immunotherapy response and serum MPO level in patients with CRC can provide useful data to predict the efficacy of breakthrough immunotherapy agents in cancer treatment.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

This prospective study was approved by the Ethics of Committees of Bakırköy Dr. Sadi Konuk Training and Research Hospital (approval number: 2018/351).

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